Ш

2 3

Ouick I.

## WHAT IS CLAIMED IS:

1 1. A method for assaying an activation state of a platelet comprising detecting 2 the catalysis of a modified prothrombinase substrate to a modified prothrombinase product by a prothrombinase which is associated with the platelet. 3 1 2. The method of claim 1 wherein the detection of the catalysis of a modified 2 prothrombinase substrate comprises detecting the production of modified thrombin. 3. The method of claim 1 wherein detecting the catalysis of a modified <u>l</u> 1 prothrombinase substrate comprises detection of modified thrombin catalytic activity. 1 4. The method of claim 1 wherein the prothrombinase enzyme comprises factor Xa, factor Va and one or more members selected from the group consisting of a PS:PC 2 vesicle and a platelet. 5. The method of claim 1 wherein the modified prothrombinase substrate 1 comprises prothrombin which is chemically derivatized by the addition of one or more 2 3 chemical groups selected from the group consisting of an acyl group, an acetyl group, a succinyl group, a maleyl group, a polyethylene glycol group, an acetylated polyethylene 4 5 glycol group, a pyridoxal 5'-phosphate group and a dichlorotriazinylaminofluorescinyl group. 1 6. The method of claim 5 wherein the modified prothrombinase substrate 2 comprises prothrombin which is chemically derivatized by the addition of an acetyl group 3 wherein the acetyl group is donated by sulfo-N-succinimidyl acetate. 1 7. The method of claim 1 wherein the modified prothrombinase substrate is a

product of an allele of a prothrombin gene selected from the group consisting of Metz and

1	8. The method of claim 2 wherein the detection of modified thrombin
2	comprises an assay selected from the group consisting of a Western blot, an Enzyme Linked
3	ImmunoSorbent Assay, an immunodiffusion assay, a surface plasmin resonance assay, and a
4	fluorescence proximity assay.
1	9. The method of claim 3 wherein the detection of modified thrombin
2	catalytic activity comprises detecting fibrin.
1	10. The method of claim 3 wherein the detection of modified thrombin
<b>4 2</b> ⊒	catalytic activity comprises detecting fibrinogen.
≝ ≝ 1	11. The method of claim 3 wherein the detection of modified thrombin
1 2 1 1 2 2 T 1 T 2 T T T T T T T T T T	catalytic activity comprises detecting cleavage of a peptide.
<b>]</b> 1	12. The method of claim 11 wherein the peptide is glycyl-L-prolyl L-arginine
2	wherein the amino terminal end of the peptide is linked to a tosyl group and the carboxyl
≟ 3 ∐	terminal end of the peptide is linked to a p-nitroanalide group.
1	13. A kit for assaying an activation state of a platelet comprising:
2	(a) a modified prothrombinase substrate; and
3	(b) a prothrombinase product assay.
1	14. The kit according to claim 13 wherein the prothrombinase product assay is
2	selected from the group consisting of a Western blot, an Enzyme Linked ImmunoSorbent
3	Assay (ELISA), an immunodiffusion assay, a surface plasmin resonance assay, a
4	chromogenic peptide cleavage assay, a polyacrylamide gel electrophoresis analysis, and a
5	fluorescence proximity assay.

1	15. The kit of claim 13 wherein the modified prothrombinase substrate is
2	prothrombin which is chemically derivatized by the addition of one or more chemical groups
3	selected from the group consisting of an acyl group, an acetyl group, a succinyl group, a
4	maleyl group, a polyethylene glycol group, an acetylated polyethylene glycol group, a
5	pyridoxal 5'-phosphate group and a dichlorotriazinylaminofluorescinyl group.
1	16. The kit of claim 13 wherein the modified prothrombinase substrate is a
2	product of an allele of a prothrombin gene selected from the group consisting of Metz and
3	Quick I
å 4	
1	17. The kit of claim 13 wherein the prothrombinase product assay comprises
1 1 2	reagents for a chromogenic peptide cleavage assay wherein the reagents comprise a peptide
<u>.</u> 3	having a sequence cleaved by thrombin.
<b>j</b> 1	18. The kit of claim 17 wherein the peptide is glycyl-L-prolyl L-arginine
<u>.</u> 2	wherein the amino terminal end of the peptide is crosslinked to a tosyl group and the carboxyl
<u> </u>	terminal end of the peptide is crosslinked to a p-nitroanalide group.
r i	
1	19. The kit of claim 13 further comprising one or more reagents selected from
2	the group consisting of human α-thrombin, calcium ionophore A23187, factor Xa, Sulfo-N-
3	succinimidyl acetate, factor Va and phospholipid vesicles comprising phosphatidylserine and
4	phosphatidylcholine.
1	20. The kit of claim 13 further comprising one or more components selected
2	from the group consisting of a glass vial, a microtiter plate, water and a syringe.